CLAIMS

1. A method for producing a molecular array which method comprises immobilising to a solid phase a plurality of molecules at a density which allows individual immobilised molecules to be individually resolved, wherein each molecule in the array is spatially addressable and the identity of each molecule is known or determined prior to immobilisation.

2. A method according to claim 1 wherein the molecules are applied to the solid phase by a method selected from printing, electronic addressing, *in situ* light-directed synthesis, ink jet synthesis or physical masking.

3. A method according to claim 2 wherein the molecules are applied to the solid phase by printing of dilute solutions.

4. A method for producing a molecular array which method comprises:

   (i) providing a molecular array comprising a plurality of molecules immobilised to a solid phase at a density such that individual immobilised molecules are not capable of being individually resolved; and

   (ii) reducing the density of functional immobilised molecules in the array such that remaining individual functional immobilised molecules are capable of being individually resolved;

   wherein each individual functional molecule in the resulting array is spatially addressable and the identity of each molecule is known or determined prior to the density reduction step.

5. A method according to claim 4 wherein the density of functional molecules is reduced by cleaving all or part of the molecules from the solid phase.

6. A method according to claim 4 wherein the density of functional molecules is reduced by functionally inactivating the molecules *in situ*.

7. A method according to claim 4 wherein the density of functional molecules is reduced by labelling some of the plurality of molecules such that individual immobilised labelled molecules are capable of being individually resolved.

8. A method according to any one of the preceding claims wherein the immobilised molecules are present within discrete spatially addressable elements.

9. A method according to claim 8 wherein the structure of molecules present in each discrete spatially addressable element is known and unintended structures are substantially absent.

10. A method according to claim 8 wherein a plurality of molecular species are present within one or more elements and each molecular species in an element can be distinguished from other molecular species in the element by means of a label.

11. A method according to any one of the preceding claims wherein the plurality of molecules which are capable of being individually resolved are capable of being resolved by optical means.

12. A method according to any one of the preceding claims wherein the plurality of molecules which are capable of being individually resolved are capable of being resolved by scanning probe microscopy.

13. A method according to any one of claims 1 to 12 wherein the molecules are attached to the solid phase at a single defined point.

14. A method according to any one of claims 1 to 12 wherein the molecules are attached to the solid phase at two or more points.

15. A method according to any preceding claim, wherein the molecules comprise a detectable label.

16. A method according to claim 15 wherein the label can be read by optical methods.

17. A method according to claim 15 or claim 16 wherein the label is a single fluorescent molecule, nanoparticle or nanorod, or a plurality of fluorescent molecules, nanoparticles or nanorods.

18. A method according to claim 15 where the label can be read by SPM.

19. A method according to claim 18 wherein the label is a non-fluorescent molecule, nanoparticle or nanorod.

20. A method according to any one of claims 1 to 19 wherein the molecules are selected from defined chemical entities, oligonucleotides, polynucleotides, peptides, polypeptides, conjugated polymers, small organic molecules or analogues, mimetics or conjugates thereof.

21. A method according to claim 20 wherein the molecules are cDNAs and/or genomic DNA.

22. A method according to any one of the preceding claims wherein the immobilised molecules are present within discrete spatially addressable elements and each element comprises a distinct spatially addressable microelectrode or nanoelectrode.

23. A method according to claim 22 wherein said electrodes are formed of conducting polymers.

24. A method according to claim 23 wherein said electrodes are produced by a method selected from inkjet printing, soft lithography, nanoimprint lithography/lithographically induced self assembly, VLSI methods and electron beam writing.

25. A method according to any one of claims 1 to 24 wherein the immobilised molecules are immobilised onto a single electrode.

26. A method according to any one of claims 22 to 25 wherein the electrode(s) transduce a signal when a target molecule binds to an immobilised molecule present in the same element as an electrode.

27. A molecular array obtained by the method of any one of the preceding claims.

28. Use of a molecular array in a method of identifying one or more target molecules in a sample, which molecular array comprises a plurality of molecules immobilised to a solid phase at a density which allows individual immobilised molecules to be individually resolved, wherein each individual immobilised molecule in the array is spatially addressable and the identity of each immobilised molecule is known or encoded.

29. Use according to claim 28 wherein said method comprises contacting the array with the sample and interrogating one or more individual immobilised molecules to determine whether a target molecule has bound.

30. Use according to claim 29 wherein substantially all of the immobilised molecules are interrogated.

31. Use according to any one of claims 28 to 30 wherein interrogation is by an optical method.

32. Use according to claim 35 wherein the optical method is selected from far-field optical methods, near-field optical methods, epi-fluorescence spectroscopy, scanning confocal microscopy, two-photon microscopy, total internal reflection microscopy,

33. Use according to claim 36 where pulsed laser excitation illumination is coupled with Time-correlated single molecule counting (TCSPC) or synchronised time gating.

34. Use according to any one of claims 28 to 30 wherein interrogation is by scanning probe microscopy or electron microscopy.

35. Use according to any one of claims 28 to 34 wherein a physicochemical property of the immobilised molecules is determined, such as shape, size, mass, hydrophobicity or charge.

36. Use according to any one of claims 28 to 34 wherein an electromagnetic, electrical, optoelectronic and/or electrochemical property of the immobilised molecules is determined.

37. Use according to any one of claims 29 to 34 wherein a characteristic of a complex between an immobilised molecule and a target molecule is determined.

38. Use according to any one of claims 28 to 37 wherein the immobilised molecules are of the same chemical class as the target molecules.

39. Use according to any one of claims 28 to 37 wherein the immobilised molecules are of a different chemical class to the target molecules.

40. Use according to any one of claims 28 to 37 wherein the target molecules are genomic DNA or reduced complexity representations thereof.

41. Use according to claim 40 wherein complexity is reduced by fragmenting the target and pre-hybridising it to C_0t=1 DNA

42. Use according to claim 40 or claim 41 wherein the genomic DNA undergoes whole genome amplification prior to analysis.

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43. Use according to any one of claims 28 to 37 wherein the target molecules are mRNA or cDNA.

44. Use of a molecular array as defined in claim 28 in genetic analysis, gene expression studies, identifying one or more molecules in the array which interact with a molecular target or in the detection or typing of single nucleotide polymorphisms in a sample of nucleic acids, haplotyping or sequencing.

45. Use of a molecular array as defined in claim 32 wherein the immobilised molecules of the array and the target molecules are nucleic acids and the contacting step takes place under conditions which allow hybridisation of the immobilised molecules to the target molecules.

46. Use according to claim 45 wherein hybridisation of a target nucleic acid to an immobilised nucleic acid is detected by means of primer extension from the resulting complex.

47. Use according to claim 45 wherein observation of successive tagged monomer base additions enables sequencing by synthesis.

48. Use according to claim 46 or claim 47, wherein the enzyme Apyrase is used to reduce incorporation 3' end mismatch bases.

49. Use according to claim 45 wherein hybridisation of a target nucleic acid to an immobilised nucleic acid is detected by means of hybridisation of nucleic acid probes to the target nucleic acid/immobilised nucleic acid complex.

50. Use according to claim 49 wherein the probes are differentially labelled.

51. Use according to claim 47 wherein hybridisation of a target nucleic acid to an immobilised nucleic acid is detected by means of ligation of nucleic acid probes to the target nucleic acid/immobilised nucleic acid complex.

52. Use according to claim 47 wherein observation of successive ligations with tagged oligonucleotides leads enables sequencing by synthesis.

53. Use according to any one of claims 28 to 52 wherein the array is contacted with two or more populations of target molecules.

54. Use according to claim 53 wherein each population of target molecules is differentially labelled.

55. A method for typing single nucleotide polymorphisms (SNPs) and mutations in nucleic acids, comprising the steps of:

a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more polymorphisms, said repertoire being presented such that molecules in said repertoire may be individually resolved;

b) exposing the sample to the repertoire and allowing nucleic acids present in the sample to hybridise to the probes at a desired stringency and optionally to be processed by enzymes;

c) detecting individual reacted nucleic acid molecules after optionally eluting the unreacted nucleic acids from the repertoire.

56. A method according to claim 55, wherein the repertoire is arrayed on a solid phase.

57. A method according to claim 56, wherein said array is an array according to claim 27.

58. A method according to any one of claims 55 to 57, wherein the sample is exposed to a second repertoire of probes, which probes bind to one or more molecules of the sample at a different position to the probes of the first repertoire.

59. A method according to claim 58, wherein said first and second repertoires are differentially labelled.

60. A method for determining the complete or partial sequence of a target nucleic acid, comprising the steps of:

a) providing a first set of probes complementary to one or more nucleic acids present in a sample, said first set of probes being presented such that arrayed molecules may be individually resolved;

b) hybridising a sample comprising a target nucleic acid to the first set of probes;

c) hybridising one or more further probes of defined sequence to the target nucleic acid; and

d) detecting the binding of individual further probes to the target nucleic acid.

e) and detecting the approximate distance separating each probe or the order of each probe

61. A method according to claim 60, wherein the first set of probes is a repertoire of probes.

62. A method according to claim 61, wherein the repertoire is arrayed on a solid phase.

63. A method according to claim 62, wherein the target nucleic acids are captured to the solid phase at one or more points.

64. A method according to any one of claims 60 to 63, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.

65. A method according to claim 64, wherein said array is an array according to claim 27.

66. A method according to any one of claims 60 to 65, wherein the probes are differentially labelled.

67. A method for determining the number of sequence repeats in a sample of nucleic acid, comprising the steps of:

a) providing one or more probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more sequence repeats, said probes being complementary to a sequence flanking one end of the repeats, said probes being presented such that molecules may be individually resolved;

b) contacting the nucleic acids with labelled probes complementary to units of said sequence repeats and a differentially labelled probe complementary to the flanking sequence at the other end of the targeted repeats ;

c) contacting the complex formed in b) with probes in a); and

d) determining the number of repeats present on each sample nucleic acid by individual assessment of the number of labels incorporated into each molecule and only counting those molecules to which the differentially labelled probe complementary to the flanking sequence is also associated with.

68. A method according to claim 67, wherein the repertoire is arrayed on a solid phase.

69. A method according to claim 67 or claim 68, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.

70. A method according to claim 69, wherein said array is an array according to claim 27.

71. A method for analysing the expression of one or more genes in a sample, comprising the steps of:

a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, said repertoire being presented such that molecules may be individually resolved;

b) hybridising a sample comprising said nucleic acids to the probes; and

c) determining the nature and quantity of individual nucleic acid species present in the sample by counting single molecules which are hybridised to the probes.

72. A method according to claim 71, wherein the repertoire is arrayed on a solid phase.

73. A method according to claim 71 or claim 72, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.

74. A method according to claim 73, wherein said array is an array according to claim 27.

75. A method according to any one of claims 71 to 74, wherein the repertoire comprises a plurality of probes of each given specificity.

76. A method for typing single nucleotide polymorphisms (SNPs) and mutations in nucleic acids, comprising the steps of:

a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more polymorphisms;

b) arraying said repertoire such that each probe in the repertoire is resolvable individually;

c) exposing the sample to the repertoire and allowing nucleic acids present in the sample to hybridise to the probes at a desired stringency and optionally be processed by enzymes such that hybridised/processed nucleic acid/probe pairs are detectable;

d) eluting the unhybridised nucleic acids from the repertoire and detecting individual hybridised/processed nucleic acid/probe pairs;

e) analysing the signal derived from step (d) and computing the confidence in each detection event to generate a PASS table of high-confidence results; and

f) displaying results from the PASS table to assign base calls and type polymorphisms present in the nucleic acid sample.

77. A method according to 76 wherein step (e) involves analysing the signal from step (d) and computing in each detection event a FAIL table of low confidence results and using this table to inform primer and assay design.

78. A method according to claim 76 or claim 77 where the process is iterated for sequencing by synthesis.

79. A method according to claim 76, wherein confidence in each detection event is computed in accordance with **Table 1**.

80. A method according to claim 76 or claim 77, wherein detection events are generated by labelling the sample nucleic acids and/or the probe molecules, and imaging said labels on the array using a detector.

81. A method according to any one of claims 55 and 76-80 where the SNPs that are probed are tags for a haplotype block or a region of linkage disequilibrium.

82. A method of obtaining allele frequencies by single molecule counting of pooled DNA.

83. A method according to claim 82 wherein obtained allele frequencies are used in association studies or other genetic methods.

84. A method according to any one of claims 76 to 83 where probe and/or target acts as a primer or ligation substrate.

85. A method according to any one of claims 76 to 80 wherein the probe and or target is enzymatically processed by ligases or polymerases or thermophilic varieties thereof or re-engineered/shuffled varieties thereof.

86. A method according to any one of claims 76 to 85 wherein the probe forms secondary structures which facilitate or stabilise hybridisation or improve mismatch discrimination.

87. A method for determining the sequence of all or part of a target nucleic acid molecule which method comprises:

(i) immobilising the target molecule to a solid phase at two or more points such that the molecule is substantially horizontal with respect to the surface of the solid phase;

(ii) straightening the target molecule, during or after immobilisation;

(iii) contacting the target molecule with a nucleic acid probe of known sequence; and

(iv) determining the position within the target molecule to which the probe hybridises.

88. A method according to claim 87 wherein the target molecule is contacted with a plurality of probes.

89. A method according to claim 88 wherein each probe is labelled with a different detectable label.

90. A method according to claim 87 or 88 wherein the target molecule is contacted sequentially with each of the plurality of probes.

91. A method according to claim 90 wherein each probe is removed from the target molecule prior to contacting the target molecule with a different probe.

92. A method according to claim 88 or 89 wherein the target molecule is contacted with all of the plurality of probes substantially simultaneously.

93. A method according to claim 91 wherein the probes are removed by heating, modifying the salt concentration or pH, or by applying an appropriately biased electric field.

94. A method or use according to any one of claim 28 to 93 wherein the target is substantially a double stranded molecule and is probed by strand invasion using PNA or LNA.

95. A method according to any one of claims 97 to 94 wherein the target nucleic acid molecule is a double-stranded molecule and is derived from a single-stranded nucleic acid molecule of interest by synthesising a complementary strand to said single-stranded nucleic acid.

96. A method or use according to any one of claims 28 to 94 wherein the target molecule is substantially single stranded and is made accessible to hybridisation by elongation or stretching out.

97. A method or use according to any one of claims 28 to 96 wherein a plurality of target molecules are analysed simultaneously.

98. A method for determining the sequence of all or part of a target single-stranded nucleic acid molecule which method comprises:

(i) immobilising the target molecule to a solid phase at two or more points such that the molecule is substantially horizontal with respect to the surface of the solid phase;

(ii) straightening the target molecule, during or after immobilisation;

(iii) contacting the target molecule with a plurality of nucleic acid probes of known sequence, each probes being labelled with a different detectable label; and

(iv) ligating bound probes to form a complementary strand.

99. A method according to claim 98 wherein prior to step (iv), any gaps between bound probes are filled by polymerisation primed by said bound probes.

100. A method according to any one of claims 87 to 99 wherein the solid phase is a bead or particle.

101. A method according to any one of claims 87 to 100 wherein the solid phase is a substantially flat surface.

102. A method for arraying a plurality of nucleic acid molecules which method comprises:

(i) contacting the plurality of nucleic acid molecules with a plurality of probes, each probe being labelled with a tag which indicates uniquely the identity of the probe, such that each molecule can be identified uniquely by detecting the probes bound to the molecule and determining the identity of the corresponding tags;

(ii) immobilising the plurality of nucleic acid molecules randomly to a solid substrate; and optionally

(iii) horizontalising and straightening the molecules, during or after immobilisation.

103. A method according to claim 102 wherein the plurality of nucleic acid molecules are immobilised at a density such that individual immobilised molecules in the sample can be individually resolved.

104. A method according to any one of claims 102 to 103 wherein the solid phase is a substantially flat solid substrate or a bead/particle/rod/bar.

105. An array produced by the method of any one of claims 102 to 104.

106. A method for identifying and/or characterising one or more molecules of a plurality of molecules present in a sample which method comprises:

(i) producing a molecular array by a method comprising immobilising to a solid phase a plurality of molecules present in a sample, wherein the plurality of molecules are immobilised at a density such that individual molecules in the sample can be individually resolved; and

(ii) identifying and/or characterising one or more molecule immobilised to the array by a method comprising contacting the immobilised molecules with a plurality of encoded probes;

wherein each probe is encoded by virtue of being labelled with a tag which indicates uniquely the identity of the probe, such that an immobilised molecule can be identified uniquely by detecting the probes bound to the molecule and determining the identity of the corresponding tags.

107. A method according to claim 106 wherein the tagged probes are produced using combinatorial chemistry.

108. A method according to claim 106 wherein the tag is selected from a nanoparticle, a nanorod and a quantum dot.

109. A method according to any one of claims 106 to 108 wherein each tag comprises multiple molecular species.

110. A method according to any one of claims 106 to 109 wherein the tags are detectable by optical means.

111. A method according to claim 106 wherein the tags are particulate and comprise surface groups.

112. A method according to claim 106 wherein the tags are particulate and encase detectable entities.

113. A method according to any one of claims 106 to 112 wherein tags can be detected and distinguished by scanning probe microscopy.

114. A method according to any one of claims 106 to 113 wherein the solid substrate is selected from the group consisting of a bead, a particle, a rod and a bar.

115. A method according to any one of claims 106 to 114 wherein the solid phase comprises channels or capillaries within which the molecules are immobilised.

116. A method according to any one of claims 106 to 115 wherein the solid phase comprises a gel.

117. A biosensor comprising a molecular array according to any one of claims 27 or 105.

118. An integrated biosensor comprising a molecular array according to claim 117, an excitation source, a dectector, such as a CCD and, optionally, signal processing means.

119. A biosensor according to claim 117 or 118 wherein the biosensor comprises a plurality of elements, each element containing distinct molecules, such as probe sequences.

120. A biosensor according to claim 119 wherein each element is specific for the detection of a different target, such as different pathogenic organisms.

121. A biosensor according to any one of claims 117 to 120 wherein the molecular array is formed on an optical fibre or waveguide.

122. A method according to claim 106 in which the plurality of probes are labeled with a tag which indicates uniquely the identity of the probe.

123. A method according to any preceding claim in which the plurality of tagged probes are hybridized substantially simultaneously or in groups of probes.

124. A method according to any preceding claim in which probes are grouped according to their Tm.

125. A method according to claim 106, in which each of the plurality of labeled probes are successively hybridized to the immobilized nucleic acid and a record of those that hybridise to each molecule can be used to identify or re-assemble the sequence of the immobilized molecule.

126. A method for determining haplotypes by probing single molecules immobilised on a solid phase in a spatially addressable manner.

127. A method according to claim 126 for haplotyping in which successive SNP sites are probed with different labels.

128. A method for haplotyping in which the first SNP is defined by the address of array element that binding occurs to and subsequent SNPs are defined by different labels.

129. A method for haplotyping on arrays, where first SNP is defined by address on array and subsequent SNPs are identified by solution probes.

130. A method for haplotyping on array captured and horizontalised and/or linearises DNA, where first SNP is defined by address on array and subsequent SNPs are identified by solution probes.

131. A method according to 130 where two different labels are used to distinguish members of the biallelic probe set and each successive SNP is identified by its position along the molecule.

132. A method according to 131 where errors are computed according to expected position of binding of probes along molecule.

133. A method where a population of molecules is analysed and the haplotypes are computed according to the consensus of signals from single molecules.

134. A method according to any one of claims 126-132 and 44, 47, 52 and 78 in which haplotype frequencies can be determined.

135. A method according to 132 and sequencing claims where markers are added to aid position SNP sites/or position of target binding.

136. A method according to any one of the preceding claims, wherein the probe is labelled or marked and signal after target binding or assay is only deemed real when it is co-incident with the label(s) or mark(s) on the probe.